APPENDIX B

Taxonomy and Metabolism of *Lactobacillus*

2

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Introduction

Today taxonomy is often based on a polyphasic approach (Vandamme *et al.*, 1996a), which involves genotypic and phenotypic methods. Pure historically phenotypic methods have dominated the identification and classification schemes of the lactic acid bacteria and the lactobacilli in particular. Today, 16S RNA sequencing has become the method of choice, not only because of its high degree of portability, but equally important, because of the availability of a large database of reference sequences. In a polyphasic approach, which involves both genotypic and phenotypic methods, one may encounter differences between, for example, the phylogenetic tree revealed by 16S RNA sequencing and the phenotypic groups based on fermentation profiles and metabolite production.

It would be wrong, however, to limit all classification schemes to the phylogenetic groupings based on 16S rRNA sequences exclusively. 16S rRNA suffers from considerable disadvantages, as will be shown below. A polyphasic approach that involves a balanced use of multiple genotypic and phenotypic methods will always yield a more balanced and reliable result. As sequencing becomes more and more accessible and cheaper, the role of a single molecule such as 16S rRNA, will tend to fade. The sequencing of a variety of individual (household) genes will diversify and broaden the taxonomic views, with the sequencing of the complete genome of many organisms as a feasible option within a few years.

Today, however, 16S rRNA in combination with DNA–DNA hybridizations is still the reference method. Therefore, the taxonomic discussion in this chapter will be based on a neighbour joining tree, obtained with the 16S rRNA of the *Lactobacillus* species known at the end of May 2008.

In the section 'Metabolism' below we describe the remarkable variation of catabolic activities within the genus *Lactobacillus*. This variation, together with the fact that lactobacilli are generally considered 'safe', has been the basis of their very broad use in food applications (Vankerckhoven *et al.*, 2008; Huys *et al.*, 2006). The active application of living microorganisms in foods requires proper labelling (Temmerman *et al.*, 2004) which makes proper identification, based on stable and reliable classification schemes, extremely important.

Metabolism

The first essential step in food fermentations is the catabolism of carbohydrates by the lactic acid bacteria. Lactic acid bacteria as a group exhibit an enormous capacity to degrade different carbohydrates and related compounds. The main end product is lactic acid (>50% of sugar carbon). It should be noticed, however, that lactic acid bacteria adapt to various conditions and change their metabolism accordingly. This may lead to significantly different end product patterns (Table 2.1).

The taxonomy of lactic acid bacteria for many decades heavily relied on the type of sugar fermentation. In order to deal with the large number of species being described, Orla-Jensen (1919, 1942, 1943) proposed a classification

Table 2.1 Historical subdivision of the genus *Lactobacillus* according to the type of fermentation

	I: Homofermentative	II: Heterofermentative
Glucose fermented to lactic acid	≥ 85%	50%
Formation of CO_2, acetic acid and ethanol	–	+
CO_2 formed from glucose	–	+
Thiamine required for growth	–	+
Fructose diphosphate aldolase	+	–

Orla-Jensen, 1919	*'Thermobacterium'* Obligate homofermentative	*'Streptobacterium'* Facultative heterofermentative	*'Betabacterium'* Obligate heterofermentative
van den Hamer, 1960			
Fructose-1,6-bisphosphate aldolase	+	+	–
Glucose-6-phosphate dehydrogenase	–	+	+
6-Phosphogluconate dehydrogenase	–	+	+
Rogosa, 1970	IA	IB	II
Growth at 45°C	+	d	
Growth at 15°C	–	d	
Ribose fermented	–	+	+
CO_2 from gluconate	–	+	+

Rogosa, 1974	II	III
Acidophilic	–	+
Ethanol tolerant	–	+
Most carbohydrates fermented	+	–

of lactic acid bacteria, which was based on morphology, temperature range of growth, nutritional characteristics, carbon sources utilization and agglutination effects. Orla-Jensen differentiated three major groups. The first group contained *Thermobacterium*, *Streptobacterium* and *Streptococcus*, which were all catalase negative and produce mainly lactic acid besides traces of other by-products. The second group contained *Betabacterium* and *Betacoccus*, which also lack catalase but as a rule formed detectable amounts of gas and other by-products, besides lactic acid. The third group consisting of *Microbacterium* and *Tetracoccus* show a positive catalase reaction.

In 1960, van den Hamer showed that representatives of *Betabacterium* did not posses fructose-1,6-bisphosphate aldolase, in contrast to *Thermobacterium* and

Table 2.1 *continued*

	I: Homofermentative		II: Heterofermentative	
Sharpe, 1979			IIA	IIB
Aerobic species	*Lactobacillus acidophilus*	*Lactobacillus casei*	*Lactobacillus brevis*	*Lactobacillus fructivorans*
	Lactobacillus delbrueckii	*Lactobacillus coryniformis*	*Lactobacillus buchneri*	*Lactobacillus hilgardii*
	Lactobacillus helveticus	*Lactobacillus curvatus*	*Lactobacillus confusus*	
	Lactobacillus jensenii	*Lactobacillus homohiochii*	*Lactobacillus fermentum*	
	Lactobacillus salivarius	*Lactobacillus plantarum*	*Lactobacillus viridescens*	
		Lactobacillus yamanshiensis		
Anaerobic species	*Lactobacillus ruminis*			
	Lactobacillus vitulinus			

Kandler and Weiss, 1986	Group I	Group II	Group III
Hexose almost exclusively to lactic acid	+	+	–
Hexose fermented to lactic-, acetic acid, ethanol, CO_2	–	–	+
Lactic-, acetic-, formic acid, ethanol under glucose limitation	–	d	+
Pentose phosphoketolase	–	+	+
Gluconate fermented	–	+	+

d, strain dependent; *L.*, *Lactobacillus*.

Streptobacterium. These findings supported the discrimination of three physiological groups: (i) the obligately homofermentative lactobacilli, lacking both glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase (*Thermobacterium*), (ii) the facultatively homofermentative lactobacilli, having both dehydrogenases, but degrading glucose preferably via the Embden–Meyerhof–Parnas pathway (*Streptobacterium*), and (iii) the obligately heterofermentative lactobacilli, lacking fructose-1,6-bisphosphate-aldolase (*Betabacterium*). *Thermobacterium*, *Streptobacterium* and *Betabacterium* were considered to be the three subgenera within the genus *Lactobacillus*.

The subdivision of lactobacilli into three major fermentation groups for taxonomic reasons was maintained until the late 1970s (Table 2.1). The development and application of advanced molecular techniques brought new insights in the taxonomy of the genus, which is meanwhile considered the most heterogeneous among the lactic acid bacteria, with currently 113 different species described (Table 2.2). However, for practical rea-

Table 2.2 List of *Lactobacillus* species with some details on their original description and their current taxonomic status. The table also contains some information on their respective phylogenetic position, metabolism and peptidoglycan type

Number	Genus	Species	Subspecies	References	Curr
1	*Lactobacillus*	*acetotolerans*		Entani *et al.* 1988	
2	*Lactobacillus*	*acidifarinae*		Vancanneyt *et al.* 2005b	
3	*Lactobacillus*	*acidipiscis*		Tanasupawat *et al.* 2000	
4	*Lactobacillus*	*acidophilus*		(Moro 1900) Hansen and Mocquot 1970; Johnson *et al.* 1980	
5	*Lactobacillus*	*agilis*		Weiss *et al.* 1981,1982	
6	*Lactobacillus*	*algidus*		Kato *et al.* 2000	
7	*Lactobacillus*	*alimentarius*		(ex Reuter 1970) Reuter 1983a,b	
8	*Lactobacillus*	*amylolyticus*		Bohak *et al.* 1998, 1999	
9	*Lactobacillus*	*amylophilus*		Nakamura and Crowell 1979,1981	
10	*Lactobacillus*	*amylotrophicus*		Naser *et al.* 2006c	
11	*Lactobacillus*	*amylovorus*		Nakamura 1981	
12	*Lactobacillus*	*animalis*		Dent and Williams 1982, 1983	
13	*Lactobacillus*	*antri*		Roos *et al.* 2005	
14	*Lactobacillus*	*apodemi*		Osawa *et al.* 2006	
	Lactobacillus	*"arizonensis"*		Swezey *et al.* 2000	*Lac* *et a*
15	*Lactobacillus*	*aviarius*		Fujisawa *et al.* 1984,1985	
		aviarius	*araffinosus*	Fujisawa *et al.* 1984,1986	
		aviarius	*aviarius*	Fujisawa *et al.* 1984,1986	
	Lactobacillus	*"backi"*		Bohack *et al.*, 2006	No
	Lactobacillus	*"bavaricus"*		Stetter and Stetter 1980	*Lac* Ca
	Lactobacillus	*"bifermentans"*		(ex Pette and van Beynum 1943) Kandler *et al.* 1983b,c	
16	*Lactobacillus*	*brevis*		(Orla-Jensen 1919) Bergey *et al.* 1934	
17	*Lactobacillus*	*buchneri*		(Henneberg 1903) Bergey *et al.* 1923	
	Lactobacillus	*"bulgaricus"*		(Orla-Jensen 1919) Rogosa and Hansen 1971	*Lac* *bu*
18	*Lactobacillus*	*camelliae*		Tanasupawat *et al.* 2007	
	"Lactobacillus"	*"canis"*		Shaw and Harding 1985, 1986	*Ca* (*Co*
19	*Lactobacillus*	*casei*		(Orla-Jensen 1916) Hansen and Lessel 1971	
	Lactobacillus	*"casei"*	*"alactosus"*	Mills and Lessel 1973	*La* *pa*
	Lactobacillus	*casei*	*casei*	(Orla-Jensen 1916) Hansen and Lessel 1971	*Se*
	Lactobacillus	*"casei"*	*"pseudoplantarum"*	Abo-Elnaga and Kandler 1965b	*La* *pa*
	Lactobacillus	*"casei"*	*"rhamnosus"*	Hansen 1968	*La* *et*
	Lactobacillus	*"casei"*	*"tolerans"*	Abo-Elnaga and Kandler 1965b	*La* *to*
20	*Lactobacillus*	*catenaformis*		(Eggerth 1935) Moore and Holdeman 1970	
	Lactobacillus	*"cellobiosus"*		Rogosa *et al.* 1953	*La* (*D*
21	*Lactobacillus*	*celli*		Vela *et al.* 2008	
22	*Lactobacillus*	*coleohominis*		Nikolaitchouk *et al.* 2001	
23	*Lactobacillus*	*composti*		Endo and Okada 2007a	
24	*Lactobacillus*	*collinoides*		Carr and Davies 1972	

Current name (reference)	Phylogenetic group	Metabolism type §	Mol% G + C content	Peptidoglycan type	Lactic acid type
	acidophilus	B	35–37	Lys-D-Asp	DL
	buchneri	C	51	NA	DL
	salivarius	B	38–41	Lys-D-Asp	L
	acidophilus	A	34–37	Lys-D-Asp	DL
	salivarius	B	43–44	DAP	L
	salivarius	B	36–37	DAP	L
	plantarum	B	36–37	Lys-D-Asp	L-DL
	acidophilus	A	39	Lys-D-Asp	DL
	acidophilus	A	44–46	Lys-D-Asp	L
	acidophilus	A	43.5	NA	L
	acidophilus	A	40–41	Lys-D-Asp	DL
	salivarius	A	41–44	Lys-D-Asp	L
	reuteri	C	44–45	Lys-D-Asp	DL
	salivarius	B	38.5	L-Lys-D-Asp	L
Lactobacillus plantarum (Kostinek *et al.* 2005)	plantarum	B	48	NA	DL
	salivarius	A	39–43	Lys-D-Asp	DL
	salivarius	A	39–43	Lys-D-Asp	DL(D <15%)
	salivarius	A	39–43	Lys-D-Asp	DL
Not yet validated	NA	NA	NA	NA	NA
Lactobacillus sakei (Kagermeier-Callaway and Lauer 1995)	casei	B	41–43	Lys-D-Asp	L
	casei	B	45	Lys-D-Asp	DL
	buchneri	C	44–47	Lys-D-Asp	DL
	buchneri	C	44–46	Lys-D-Asp	DL
Lactobacillus delbrueckii subsp. *bulgaricus* (Weiss *et al.* 1983b)	acidophilus	A	49–51	Lys-D-Asp	D
	casei	A	51.9	Lys-D-Asp	L
Carnobacterium maltaromaticus (Collins *et al.* 1987)	casei	B	45–47	Lys-D-Asp	L
Lactobacillus paracasei subsp. *paracasei* (Collins *et al.* 1989b)	casei	B	45–47	Lys-D-Asp	L
See text for further explanation	casei	B	45–47	Lys-D-Asp	L
Lactobacillus paracasei subsp. *paracasei* (Collins *et al.* 1989b)	casei	B	45–47	Lys-D-Asp	L
Lactobacillus rhamnosus (Collins *et al.* 1989b)	casei	B	45–47	Lys-D-Asp	L
Lactobacillus paracasei subsp. *tolerans* (Collins *et al.* 1989b)	casei	B	45–47	Lys-D-Asp	L
	vitulinus-catenaformis	A	31–33	Lys-Ala	D
Lactobacillus fermentum (Dellaglio *et al.* 2004a)	reuteri	C	53	Orn-D-Asp	L or DL
	salivarius	B	NA	Lys-L-Ser	L
	reuteri	B	NA	mDAP	DL
	perolens	C	46	Lys-D-Asp	DL
	plantarum	B	48	no mDAP	DL

Table 2.2 *continued*

Number	Genus	Species	Subspecies	References
25	*Lactobacillus*	*concavus*		Tong and Dong 2005
	"Lactobacillus"	*confusus*		(Holzapfel and Kandler 1969) Sharpe *et al.* 1972
26	*Lactobacillus*	*coryniformis*		Abo-Elnaga and Kandler 1965b
		coryniformis	*coryniformis*	Abo-Elnaga and Kandler 1965b
		coryniformis	*torquens*	Abo-Elnaga and Kandler 1965b
27	*Lactobacillus*	*crispatus*		(Brygoo and Aladame 1953) Moore and Holdeman 1970
28	*Lactobacillus*	*curvatus*		(Troili-Petersson 1903) Abo-Elnaga and Kandler 1965b emend. Klein *et al.* 1996
		curvatus	*curvatus*	Tomani *et al.* 1996
		curvatus	*"melibiosus"*	Tomani *et al.* 1996
	Lactobacillus	*"cypricasei"*		Lawson *et al.* 2001a
29	*Lactobacillus*	*delbrueckii*		(Leichmann 1896) Beijerinck 1901
		delbrueckii	*bulgaricus*	(Orla-Jensen 1919) Weiss *et al.* 1983b,1984
		delbrueckii	*delbrueckii*	(Leichmann 1896) Beijerinck 1901
		delbrueckii	*indicus*	Dellaglio *et al.* 2005
		delbrueckii	*lactis*	(Orla-Jensen 1919) Weiss *et al.* 1983b,1984
30	*Lactobacillus*	*diolivorans*		Krooneman *et al.* 2002
	Lactobacillus	*"disidiosus"*		Vaughn, *et al.* 1949
	"Lactobacillus"	*divergens*		Holzapfel and Gerber 1983,1984
	Lactobacillus	*"durianis"*		Leisner *et al.* 2002
31	*Lactobacillus*	*equi*		Morotomi *et al.* 2002
32	*Lactobacillus*	*ferciminis*		(ex Reuter 1970) Reuter 1983a,b
33	*Lactobacillus*	*farraginis*		Endo and Okada 2007b
	Lactobacillus	*"farintoshensis"*		Simpson *et al.*2001, 2002
34	*Lactobacillus*	*fermentum*		Beijerinck 1901 emend. Dellaglio *et al.* 2004a
35	*Lactobacillus*	*fornicalis*		Dicks *et al.* 2000
36	*Lactobacillus*	*fructivorans*		Charlton *et al.* 1934
	"Lactobacillus"	*fructosus*		Kodama 1956
37	*Lactobacillus*	*frumenti*		Müller *et al.* 2000
38	*Lactobacillus*	*fuchuensis*		Sakala *et al.* 2002
39	*Lactobacillus*	*gallinarum*		Fujisawa *et al.* 1992
40	*Lactobacillus*	*gasseri*		Lauer and Kandler 1980
41	*Lactobacillus*	*gastricus*		Roos *et al.* 2005
42	*Lactobacillus*	*ghanensis*		Nielsen *et al.* 2007
43	*Lactobacillus*	*graminis*		Beck *et al.* 1988, 1989
	"Lactobacillus"	*"halotolerans"*		Kandler *et al.* 1983a,c
44	*Lactobacillus*	*hammesii*		Valcheva *et al.* 2005
45	*Lactobacillus*	*hamsteri*		Mitsuoka and Fujisawa 1987,1988
46	*Lactobacillus*	*harbinensis*		Miyamoto *et al.* 2005,2006
47	*Lactobacillus*	*hayakitensis*		Morita *et al.* 2007

Current name (reference)	Phylogenetic group	Metabolism type §	Mol% G + C content	Peptidoglycan type	Lactic acid type
	perolens	A	46–47	mDAP	DL (D 5%)
Weissella confusa (Collins *et al.* 1993)					
	casei	B	45	Lys-D-Asp	DL
	casei	B	45	Lys-D-Asp	DL (L<15%)
	casei	B	45	Lys-D-Asp	D
	acidophilus	A	35–38	Lys-D-Asp	DL
	casei	B	42–44	Lys-D-Asp	DL
	casei	B	42–44	Lys-D-Asp	DL
Lactobacillus sakei subsp. *carnosus* (Koort *et al.* 2004)	casei	B	42–44	Lys-D-Asp	DL
Lactobacillus acidipiscis (Naser *et al.* 2006b)	salivarius	B	40	NA	L
	acidophilus	A	49–51	Lys-D-Asp	D
	acidophilus	A	49–51	Lys-D-Asp	D
	acidophilus	A	49–51	Lys-D-Asp	D
	acidophilus	A	NA	NA	D
	acidophilus	A	49–51	Lys-D-Asp	D
	buchneri	C	NA	NA	NA
Lactobacillus kefiri (Marshall *et al.* 1984).	buchneri	C	NA	NA	NA
Carnobacterium divergens (Collins *et al.*, 1987)					
Lactobacillus vaccinostercus (Dellaglio *et al.* 2005)	reuteri	C	43	NA	DL
	salivarius	A	38–39	NA	DL
	plantarum	A	34–36	Lys-D-Asp	L (D<15%)
	buchneri	B	40–41	no mDAP	DL
Lactobacillus parabuchneri (Vancanneyt *et al.* 2005a)	buchneri	C	43	NA	DL
	reuteri	C	52–54	Orn-D-Asp	DL
	acidophilus	B	37	NA	DL
	buchneri	C	38–41	Lys-D-Asp	DL
Leuconostoc fructosum (Antunes *et al.* 2002)					
	reuteri	C	43–44	Lys-D-Asp	L
	casei	B	41–42	NA	L (D<40%)
	acidophilus	A	36–37	Lys-D-Asp	DL
	acidophilus	A	33–35	Lys-D-Asp	DL
	reuteri	C	41–42	L-Orn-D-Asp	DL
	salivarius	A	38	mDAP	DL
	casei	B	41–43	Lys-D-Asp	DL
Lactobacillus viridescens subsp. *halotolerans*; *Weissella halotolerans* (Collins *et al.*1993,1994)					
	buchneri	B	NA	L-Lys-D-Asp	DL
	acidophilus	B	33–35	Lys-D-Asp	DL
	perolens	B	53–54	NA	L
	salivarius	A	34	Lys-Asp	L

Table 2.2 *continued*

Number	Genus	Species	Subspecies	References
48	*Lactobacillus*	*helveticus*		(Orla-Jensen 1919) Bergey *et al.* 1925
	Lactobacillus	*"heterohiochii"*		Kitahara *et al.* 1957a,b; Momose *et al.* 1974
49	*Lactobacillus*	*hilgardii*		Douglas and Cruess 1936
50	*Lactobacillus*	*homohiochii*		Kitahara *et al.* 1957a,b
51	*Lactobacillus*	*iners*		Falsen *et al.* 1999
52	*Lactobacillus*	*ingluviei*		Baele *et al.* 2003
53	*Lactobacillus*	*intestinalis*		(ex Hemme 1974) Fujisawa *et al.* 1990
54	*Lactobacillus*	*jensenii*		Gasser *et al.* 1970
55	*Lactobacillus*	*johnsonii*		Fujisawa *et al.* 1992
	Lactobacillus	*"jugurtii"*		Orla-Jensen, 1919
56	*Lactobacillus*	*kalixensis*		Roos *et al.* 2005
	"Lactobacillus"	*kandleri*		Holzapfel and van Wyk 1982,1983
57	*Lactobacillus*	*kefiranofaciens*		Fujisawa *et al.* 1988 emend. Vancanneyt *et al.* 2004
		kefiranofaciens	*kefiranofaciens*	Fujisawa *et al.* 1988
		kefiranofaciens	*kefirgranum*	(Takizawa *et al.* 1994) Vancanneyt *et al.* 2004
	Lactobacillus	*"kefirgranum"*		Takizawa *et al.* 1994
58	*Lactobacillus*	*kefiri*		Kandler and Kunath 1983a,b
59	*Lactobacillus*	*kimchii*		Yoon *et al.* 2000
60	*Lactobacillus*	*kitasatonis*		Mukai *et al.* 2003
61	*Lactobacillus*	*kunkeei*		Edwards *et al.* 1998a,b
	Lactobacillus	*"lactis"*		(Orla-Jensen 1919) Bergey *et al.* 1934
	Lactobacillus	*"leichmannii"*		(Henneberg 1903) Bergey *et al.* 1923
62	*Lactobacillus*	*lindneri*		(ex Henneberg 1901) Back *et al.* 1996, 1997
63	*Lactobacillus*	*malefermentans*		(ex Russell and Walker 1958) Farrow *et al.* 1988,1989
64	*Lactobacillus*	*mali*		(Carr and Davies 1970) Kaneuchi *et al.* 1988
		mali	*mali*	(Carr and Davies 1970) Kaneuchi *et al.* 1988
		mali	*yamanashiensis*	Nonomura 1983, Kaneuchi *et al.* 1988
	"Lactobacillus"	*"mallaromicus"*		Miller *et al.* 1974
65	*Lactobacillus*	*manihotivorans*		Morlon-Guyot *et al.* 1998
66	*Lactobacillus*	*mindensis*		Ehrmann *et al.* 2003
	"Lactobacillus"	*"minor"*		Kandler *et al.* 1983a,c
	"Lactobacillus"	*minutus*		(Haudurov *et al.* 1937) Moore and Holdeman 1972; Olsen *et al.* 1991
67	*Lactobacillus*	*mucosae*		Roos *et al.* 2000
68	*Lactobacillus*	*murinus*		Hemme *et al.* 1980, 1982
69	*Lactobacillus*	*nageli*		Edwards *et al.* 2000
70	*Lactobacillus*	*namurensis*		Scheirlinck *et al.* 2007
71	*Lactobacillus*	*nantensis*		Valcheva *et al.* 2006
72	*Lactobacillus*	*oligofermentans*		Koort *et al.* 2005a,b

Current name (reference)	Phylogenetic group	Metabolism type §	Mol% G + C content	Peptidoglycan type	Lactic acid type
	acidophilus	A	38–40	Lys-D-Asp	DL
Lactobacillus fructivorans (Weiss *et al.* 1983a)	buchneri	C	38–40	Lys-D-Asp	DL
	buchneri	C	39–41	Lys-D-Asp	DL
	buchneri	B	35–38	Lys-D-Asp	DL
	acidophilus	A	34–35	Lys-D-Asp	L
	reuteri	C	49–50	NA	NA
	acidophilus	B	33–35	Lys-D-Asp	DL
	acidophilus	B	35–37	Lys-D-Asp	D
	acidophilus	A	33–35	Lys-D-Asp	DL
Lactobacillus helveticus (Simonds *et al.* 1971)	acidophilus	A	NA	NA	NA
	acidophilus	A	35–36	Lys-D-Asp	DL
Weissella kandleri (Collins *et al.* 1993,1994)	acidophilus	A	34–38	NA	DL
	acidophilus	A	34–38	NA	DL
	acidophilus	A	34–38	NA	DL
Lactobacillus kefiranofaciens subsp. *kefiranofaciens* (Vancanneyt *et al.* 2004)	acidophilus	A	34–38	no mDAP	DL
	buchneri	C	41–42	Lys-D-Asp	DL
	plantarum	B	35	NA	DL
	acidophilus	B	37–40	NA	DL
	buchneri	C	NA	Lys-D-Asp	L
Lactobacillus delbrueckii subsp. *lactis* (Weiss *et al.* 1983b,1984)	acidophilus	A	50	NA	NA
Lactobacillus delbrueckii subsp. *lactis* (Weiss *et al.* 1983b,1984)	acidophilus	A	51	NA	NA
	buchneri	C	35	Lys-D-Asp	DL
	plantarum	C	41–42	Lys-D-Asp	NA
	salivarius	A	32–34	DAP	L
	salivarius	A	32–34	DAP	L
	salivarius	A	32–34	mDAP	L
Carnobacterium piscicola (Mora *et al.* 2003) *Carnobacterium maltaromaticus* (Mora *et al.* 2003)	casei	A	48–49	NA	L
	plantarum	A	37–38	Lys-D-Asp	DL
Lactobacillus viridescens subsp. minor; *Weissella minor* (Collins *et al.* 1993,1994)					
Atopobium minutum (Collins and Wallbanks 1992, 1993)					
	reuteri	C	46–47	Orn-D-Asp	DL
	salivarius	B	43–44	Lys-D-Asp	L
	salivarius	A	NA	NA	DL
	buchneri	C	52	NA	DL
	plantarum	B	38.6	NA	DL
	reuteri	C	35.3–39.9	NA	DL(D 30%)

Table 2.2 *continued*

Number	Genus	Species	Subspecies	References
73	*Lactobacillus*	*oris*		Farrow and Collins 1988
74	*Lactobacillus*	*panis*		Wiese *et al.* 1996
75	*Lactobacillus*	*pantheris*		Liu and Dong 2002
76	*Lactobacillus*	*parabrevis*		Vancanneyt *et al.* 2006b
77	*Lactobacillus*	*parabuchneri*		Farrow *et al.* 1988, 1989
		parabuchneri	*parabuchneri*	Farrow *et al.* 1988, 1989
		parabuchneri	*ferintoshensis*	Vancanneyt *et al.*, 2005a
78	*Lactobacillus*	*paracasei*		Collins *et al.* 1989b
		paracasei	*paracasei*	Collins *et al.* 1989b
		paracasei	*tolerans*	(Abo-Elnaga and Kandler 1965b) Collins *et al.* 1989b
79	*Lactobacillus*	*paracollinoides*		Suzuki *et al.* 2004
80	*Lactobacillus*	*paraferraginis*		Endo and Okada 2007b
81	*Lactobacillus*	*parakefiri*		Takizawa *et al.* 1994
82	*Lactobacillus*	*paralimentarius*		Cai *et al.* 1999
83	*Lactobacillus*	*paraplantarum*		Curk *et al.* 1996
	Lactobacillus	*"pastorianus"*		Van Laer, 1892
84	*Lactobacillus*	*pentosus*		(ex Fred *et al.* 1921) Zanoni *et al.* 1987
85	*Lactobacillus*	*perolens*		Back *et al.* 1999, 2000
	"Lactobacillus"	*"piscicola"*		Hiu *et al.* 1984
86	*Lactobacillus*	*plantarum*		(Orla-Jensen 1919) Bergey *et al.* 1923
		plantarum	*argentoratensis*	Bringel *et al.* 2005
		plantarum	*plantarum*	(Orla-Jensen 1919) Bergey *et al.* 1923; Bringel *et al.* 2005
87	*Lactobacillus*	*pontis*		Vogel *et al.* 1994
88	*Lactobacillus*	*psittaci*		Lawson *et al.* 2001b
89	*Lactobacillus*	*rennini*		Chenoll *et al.* 2006a
90	*Lactobacillus*	*reuteri*		Kandler *et al.* 1980, 1982
91	*Lactobacillus*	*rhamnosus*		(Hansen 1968) Collins *et al.* 1989b
	"Lactobacillus"	*rimae*		Olsen *et al.* 1991
92	*Lactobacillus*	*rogosae*		Holdeman and Moore 1974
93	*Lactobacillus*	*rossiae*		Corsetti *et al.* 2005
94	*Lactobacillus*	*ruminis*		Sharpe *et al.* 1973
95	*Lactobacillus*	*saerimneri*		Pedersen and Roos 2004
96	*Lactobacillus*	*sakei*		Katagiri *et al.* 1934 emend. Klein *et al.* 1996
		sakei	*carnosus*	Torriani *et al.* 1996; Koort *et al.* 2004
		sakei	*sakei*	Katagiri *et al.* 1934 emend. Klein *et al.* 1996
97	*Lactobacillus*	*salivarius*		Rogosa *et al.* 1953
		salivarius	*salicinius*	Rogosa *et al.* 1953 emend. Li *et al.* 2006
		salivarius	*salivarius*	Rogosa *et al.* 1953 emend. Li *et al.* 2006
98	*Lactobacillus*	*sanfranciscensis*		(ex Kline and Sugihara 1971) Weiss and Schillinger 1984a,b
99	*Lactobacillus*	*satsumensis*		Endo and Okada 2005
100	*Lactobacillus*	*secaliphilus*		Ehrmann *et al.* 2007
101	*Lactobacillus*	*sharpeae*		Weiss *et al.* 1981,1982

Current name (reference)	Phylogenetic group	Metabolism type §	Mol% G + C content	Peptidoglycan type	Lactic acid type
	reuteri	C	49–51	Orn-D-Asp	DL
	reuteri	C	49–51	Lys-D-Asp	DL
	casei	A	52–53	NA	D
	buchneri	C	49	NA	DL
	buchneri	C	44	Lys-D-Asp	NA
	buchneri	C	44	Lys-D-Asp	NA
	buchneri	C	43	NA	DL
	casei	B	45–47	Lys-D-Asp	L
	casei	B	45–47	Lys-D-Asp	L
	casei	B	45–47	Lys-D-Asp	L
	plantarum	C	44–45	NA	D
	buchneri	B	40	no mDAP	DL (D< 70%)
	buchneri	C	41–42	NA	L
	plantarum	B	37–38	NA	NA
	plantarum	B	44–45	DAP	DL
Lactobacillus paracollinoides (Ehrmann and Vogel 2005)		C	NA	NA	NA
	plantarum	B	46–47	DAP	DL
	perolens	B	49–53	Lys-D-Asp	L
Carnobacterium piscicola (Collins *et al.*, 1987), *Carnobacterium maltaromaticus* (Mora *et al.* 2003)					
	plantarum	B	44–46	DAP	DL
	plantarum	B	44–46	NA	DL
	plantarum	B	44–46	DAP	DL
	reuteri	C	53–56	Orn-D-Asp	DL
	acidophilus	C	NA	NA	NA
	casei	B	NA	L-Lys-D-Asp	DL
	reuteri	C	40–42	Lys-D-Asp	DL
	casei	B	45–47	Lys-D-Asp	L
Atopobium rimae (Collins and Wallbanks 1992,1993)					
Taxonomic status unclear due to lack of type strain (Felis *et al.* 2004)	NA	NA	NA	NA	NA
	reuteri	C	44–45	Lys-Ser-Ala,	DL
	salivarius	A	44–47	DAP	L
	salivarius	A	42–43	DAP	DL
	casei	B			
	casei	B	42–44	NA	DL
	casei	B	42–44	NA	DL
	salivarius	A	34–36	Lys-D-Asp	L
	salivarius	A	NA	NA	NA
	salivarius	A	34–36	Lys-D-Asp	L
	buchneri	C	36–38	Lys-Ala	DL
	salivarius	A	39–41	DAP	L
	reuteri	B	48	L-Lys-D-Asp	L (D 5%)
	casei	A	53	DAP	L

Table 2.2 *continued*

Number	Genus	Species	Subspecies	References
102	*Lactobacillus*	*sliginis*		Aslam *et al.* 2006
	Lactobacillus	*"sobrius"*		Konstantinov *et al.* 2006
103	*Lactobacillus*	*spicheri*		Meroth *et al.* 2004a,b
104	*Lactobacillus*	*suebicus*		Kleynmans *et al.* 1989
	Lactobacillus	*"suntoryeus"*		Cachat and Priest 2005
105	*Lactobacillus*	*thailandensis*		Tanasupawat *et al.* 2007
	Lactobacillus	*"thermotolerans"*		Niamsup *et al.* 2003
	Lactobacillus	*"trichodes"*		Fornachon *et al.* 1949
	Lactobacillus	*"tucceti"*		Chenoll *et al.* 2006b
	"Lactobacillus"	*uli*		Olsen *et al.* 1991
106	*Lactobacillus*	*ultunensis*		Roos *et al.* 2005
107	*Lactobacillus*	*vaccinostercus*		Okada *et al.* 1979; Kozaki and Okada 1983 emend. Dellaglio *et al.*2006
108	*Lactobacillus*	*vaginalis*		Embley *et al.* 1989
109	*Lactobacillus*	*versmoldensis*		Kröckel *et al.* 2003
110	*Lactobacillus*	*vini*		Rodas *et al.* 2006
	"Lactobacillus"	*viridescens*		Niven and Evans 1957
111	*Lactobacillus*	*vitulinus*		Sharpe *et al.* 1973
	"Lactobacillus"	*"xylosus"*		Kitahara 1938
	Lactobacillus	*"yamanashiensis"*		Nonomura 1983
112	*Lactobacillus*	*zeae*		(ex Kuznetsov 1959) Dicks *et al.* 1996
113	*Lactobacillus*	*zymae*		Vancanneyt *et al.* 2005b

§Type of glucose fermentation as defined by Hammes and Vogel (1995) and Hammes and Hertel (2003): A = homofermentative, B = facultatively heterofermentative, C = obligately heterofermentative, NA = not available.

sons the genus today is still considered divided in the same three major groups, namely group I (obligately homofermentative lactobacilli), group II (facultatively homofermentative) and group III (obligately heterofermentative) (Tables 2.1 and 2.2). In addition, the accumulated knowledge on their sugar fermentation patterns created a solid basis on which further research was carried out, including other metabolic properties of the lactobacilli, such as proteolytic and lipolytic activities, which are equally important in food applications. These aspects are further discussed below.

Carbon sources metabolism in lactobacilli

Lactose fermentation

Lactose fermentation is by far the most studied disaccharide metabolism in lactic acid bacteria, since it is the major carbohydrate of milk. As shown for *Lactobacillus casei*, lactose is taken up via the phosphoenolpyruvate-dependent phosphotransferase system (PTS) and enters the cytoplasm as lactose phosphate (Chassy and Alpert, 1989). Lactose phosphate is cleaved by phospho-β-D-galactosidase (P-β-gal) to yield glucose and galactose-6-phosphate. Glucose is

Current name (reference)	Phylogenetic group	Metabolism type §	Mol% G + C content	Peptidoglycan type	Lactic acid type
	reuteri	C	44.5	L-Lys-D-Glu-L-Ala	NA
Lactobacillus amylovorus (Viljanen *et al.* 2008)	acidophilus	B	35–36	NA	DL
	buchneri	B	55	Lys-D-Asp	DL
	reuteri	C	40–41	DAP	DL
Lactobacillus helveticus (Naser *et al.* 2006a)	acidophilus	A	NA	NA	NA
	casei	A	49–50	no mDAP	DL
Lactobacillus ingluviei (Felis *et al.* 2005)	reuteri	C	49–51	no mDAP; Lys-Asp	DL
Lactobacillus fructivorans (Weiss *et al.* 1983a)	buchneri	C	NA	NA	NA
not yet validated	plantarum	A	ND	L-Lys-Gly-D-Asp	DL
Olsenella uli (Dewhirst *et al.* 2001)					
	acidophilus	A	35–36	Lys-D-Asp	DL
	reuteri	C	36–37	DAP	NA
	reuteri	C	38–41	Orn-D-Asp	NA
	plantarum	A	40–41	NA	L
	salivarius	B	39.4	L-Lys-D-Asp	DL
Weissella viridescens (Collins *et al.* 1993,1994)	vitulinus-catenaformis	A	34–37	mDAP	D
Lactococcus lactis subsp. *lactis* (Schleifer *et al.*, 1985)					
Lactobacillus mali (Kaneuchi *et al.*, 1988)	salivarius	A	32–34	mDAP	L
	casei	B	48–49	Lys-D-Asp	L
	buchneri	C	53–54	NA	DL

phosphorylated by glucokinase and metabolized through either the glycolytic pathway or the pentose phosphate pathway. Galactose-6-phosphate is metabolized through the tagatose-6-phosphate pathway (Bisset and Anderson, 1974), while the Leloir pathway is used by galactose-fermenting lactic acid bacteria, which transport galactose with a permease and which lack the galactose-PTS (Konings *et al.*, 1989). The enzyme systems of lactose-PTS and P-β-gal are generally inducible, and repressed by glucose (Kandler, 1983). An equally common way for lactic acid bacteria to metabolize lactose is by means of a lactose carrier (permease) and subsequent cleavage by β-galactosidase (β-gal) to yield glucose and galactose, which may again enter the two major pathways (McKay *et al.*, 1970; Bhowmik and Marth, 1990). Some of the thermophilic lactobacilli, such as *Lactobacillus delbrueckii* subsp. *bulgaricus*, *L. delbrueckii* subsp. *lactis* and *Lactobacillus acidophilus*, only metabolize the glucose moiety after transport of lactose and cleavage by β-gal, while galactose is excreted into the medium (Hickey *et al.*, 1986; Hutkins and Morris, 1987).

Glucose fermentation

For glucose fermentation two major pathways occur in lactic acid bacteria. The Embden–Meyerhof–Parnas pathway (glycolysis) is used by all lactic acid bacteria except leuconostocs, group III lactobacilli (obligately heterofermenative species), oenococci and weissellas. It is characterized by the formation of fructose-1,6-diphosphate (FDP), which is split by the

FDP aldolase into dihydroxyacetone-phosphate (DHAP) and glycerinaldehyde-3-phosphate (GAP). GAP (and DHAP via GAP) is then converted to pyruvate in a metabolic sequence including substrate level phosphorylation. One mole of glucose results in 2 moles of lactic acid and a net gain of 2 ATP. The glycolysis pathway is used by the *homofermentative* lactic acid bacteria. The other main fermentation pathway is the pentose phosphate pathway. The key step is the phosphokerolase split of xylulose-5-phoshate to glycerinaldehyde-3-phosphate (GAP) and acetyl-phosphate. GAP is then converted to lactate, while acetyl-phosphate to acetate and ethanol. This pathway is used by the heterofermentative lactic acid bacteria. Heterolactic fermentation gives 1 mole each of lactic acid, ethanol and CO_2 and 1 ATP per mole of glucose. It should be noted that glycolysis may lead to a heterolactic fermentation (meaning significant amounts of other end-products besides lactic acid) under certain conditions, and some lactic acid bacteria, regarded as homofermentative, use the pentose phosphate pathway when metabolizing certain substrates (Axsellson, 1998).

Among lactic acid bacteria, those found in sourdough fermentations belong mainly to the heterofermentative lactobacilli, which catabolize glucose via the pentose phosphate pathway. Under micro-aerophilic conditions, both oxygen and fructose can be used as electron acceptors. This gives rise to the formation of additional metabolites such as acetate and mannitol (Hammes and Gänzle, 1998).

Maltose fermentation

In sourdough, maltose is the most abundant fermentable carbohydrate, and hence maltose catabolism is a key process during fermentation. Microbial associations of maltose-positive and maltose-negative lactic acid bacteria strains are typical for sourdoughs dominated by *Lactobacillus sanfranciscensis* (Gobbetti, 1998). In *L. sanfranciscensis*, *Lactobacillus reuteri* and *Lactobacillus fermentum* a constitutive intracellular maltose phosphorylase catalyses the phosphorolytic cleavage of maltose, yielding glucose 1-phosphate and glucose (Vogel *et al.*, 1994). Glucose 1-phosphate is then converted by phosphoglucomutase to glucose 6-phosphate, which is further metabolized via the pentose phosphate pathway (Hammes *et al.*, 1996; Vogel *et al.*, 1999). On the other hand, hexokinase activity, which catalyses the conversion of glucose to glucose-6-phosphate, is virtually absent in cells growing exponentially in maltose-containing media, and thus the non-phosphorylated glucose becomes excreted in the medium in a molar ratio with maltose of about 1:1 (Stolz *et al.*, 1993; Gobbetti *et al.*, 1994). It has been shown, however, that no glucose accumulation occurred in the fermentation broth, and no maltose phosphorylase activity could be detected in cell extracts prepared from cells grown in the presence of both maltose and fructose, suggesting that in the presence of both maltose and fructose in the medium, induction of hexokinase activity does occur (De Vuyst *et al.*, 2003). Similarly, in experiments performed with growing cells of *L. sanfranciscensis*, no significant accumulation of glucose was observed in the medium as that reported for resting cells of *L. sanfranciscensis*, *L. reuteri*, and *Lactobacillus pontis* (Neubauer *et al.*, 1994; Stolz *et al.*, 1995a, b). It is also believed that hexokinase activity is induced in the presence of glucose or fructose in the medium (Stolz *et al.*, 1996).

Fructose fermentation

L. sanfranciscensis and *L. pontis* are able to use fructose as carbon source; however, in the presence of maltose they use it mainly as an electron acceptor and fructose is reduced to mannitol (Stolz *et al.*, 1995a; Hammes *et al.*, 1999; Wolfrum and Vogel, 1999). According to Röcken and Voysey (1995), oxygen was proved to be the preferred hydrogen acceptor for the *L. sanfranciscensis* strains. When oxygen is depleted, fructose is used as an electron acceptor (Gobbetti *et al.*, 1995; Stolz *et al.*, 1995a). Through the reduction of fructose to mannitol, extra ATP is produced via the acetate kinase reaction, and thus maltose-fructose co-metabolism yields shorter lag phase and higher growth rate and biomass production. It has been shown that at a molar ratio of 4:1 (fructose–maltose), acetic acid is the main product (Martinez-Anaya *et al.*, 1994; Gobbetti *et al.*, 1995, 2000; Stolz *et al.*, 1995a). *L. sanfranciscensis* converts stoichiometrically fructose to mannitol,

while *L. pontis* produces small amounts of lactic acid and ethanol (Hammes *et al.*, 1996).

Pentose fermentation

As far as pentose fermentation is concerned (Kandler, 1983; Posthuma *et al.*, 2002), despite some strain and species differences, group II and group III lactobacilli are pentose positive. In general, specific permeases are used to transport the sugars into the cell. The pentoses are then phosphorylated and converted by epimerases or isomerases to ribulose 5-phosphate or xylulose 5-phosphate, respectively, which can be metabolized by the lower half of the pentose phosphate pathway (Kandler, 1983).

Citrate fermentation

Citrate, which is present in many raw materials such as milk, vegetables, etc., can also serve as energy source for lactic acid bacteria. It is generally accepted that next to carbohydrates, citrate metabolism plays an important role in food fermentations. The ability of lactic acid bacteria to metabolize citrate is invariably linked to endogenous plasmid that contains the gene encoding the transporter, which is responsible for citrate uptake from the medium (Hugenholtz, 1993). Within the cell, citrate is initially converted by the citrate lyase to acetate and oxaloacetate. Oxaloacetate is then decarboxylated to pyruvate. According to the intracellular enzyme pool, pyruvate may be then converted (i) to acetyl CoA (via the pyruvate dehydrogenase complex), which leads to acetate (via the acetate kinase) and acetaldehyde/ethanol formation (via the alcohol dehydrogenase), (ii) to formate (via the pyruvate formate lyase), (iii) to alpha-acetolactate (via the acetolactate synthase), which leads to acetoin (acetolactate decarboxylate), and diacetyl and 2,3-butanediol (via the diacetyl/acetoin reductase), and finally (iv) to lactate (via the lactate dehydrogenase). The energy is mostly generated from the conversion of acetyl CoA to acetate, meaning that citrate acts as electron acceptor, resulting in a higher production of acetate and ATP probably via the acetate kinase pathway. Additional energy is produced during the initial breakdown of citrate into pyruvate (Hugenholtz, 1993). Furthermore, recent studies performed with *Lactococcus lactis* subsp. *lactis* biovar diacetylactis (Hugenholtz, 1993; Hugenholtz *et al.*, 1993) and *Leuconostoc oenos* (Marty-Teysset *et al.*, 1996) indicated that the uptake of citrate is coupled to the generation of a proton-motive force, which was shown to be strong enough to drive the additional ATP synthesis. Some of the products of citrate catabolism, such as diacetyl, acetaldehyde and acetoin, have very distinct aroma properties and influence significantly the quality of fermented foods. For instance, diacetyl determines the aromatic properties of fresh cheese, fermented milk, cream and butter (De Figueroa *et al.*, 1998). The breakdown of citrate results as well in the production of carbon dioxide, which can add to the texture of some fermented dairy products (Kimoto *et al.*, 1999).

It has been shown that several strains of *L. sanfranciscensis* are able to use citrate as electron acceptor in the presence of maltose (Stolz *et al.*, 1995a). On the other hand, co-metabolism of maltose and citrate has not been observed for *L. pontis* (Hammes *et al.*, 1996). According to Gobbetti and Corsetti (1996), during co-metabolism of maltose and citrate, lactic acid and acetic acid are initially produced, but when citrate is exhausted, lactic acid and ethanol are the main products. In all cases, maltose serves as carbon source, while citrate as electron acceptor. The production of small amounts of succinate from citrate has been also observed, indicating the presence of citrate lyase, malate dehydrogenase, fumarase and succinate dehydrogenase. A putative citric acid cycle (PCAC) for *L. casei* was recently generated, utilizing the genome sequence and metabolic flux analyses (Diaz-Muniz *et al.*, 2006). Although it was possible to construct a unique PCAC for *L. casei*, its full functionality was unknown. Therefore, the *L. casei* PCAC was evaluated utilizing end product analyses of citric acid catabolism during growth in modified chemically defined media (mCDM), and Cheddar cheese extract (CCE). Results suggest that under energy source excess and limitation in mCDM this micro-organism produces mainly L-lactic acid and acetic acid, respectively. Both organic acids were produced in CCE. Additional end products include D-lactic acid, acetoin, formic acid, ethanol, and diacetyl. Production of

succinic acid, malic acid, and butanendiol was not observed. It is thus concluded that under conditions similar to those present in ripening cheese, citric acid is converted to acetic acid, L/D-lactic acid, acetoin, diacetyl, ethanol, and formic acid. The PCAC suggests that conversion of the citric acid-derived pyruvic acid into acetic acid, instead of lactic acid, may yield two ATPs per molecule of citric acid.

Lactobacilli usually dominate the lactic acid bacteria microflora in naturally fermented sausages. The growth and metabolism of lactic acid bacteria is affected by the presence of oxygen. Usually the carbohydrates are metabolized via glycolysis. However, under certain conditions, the heterofermentative pathway is activated, resulting in undesirable flavour components, i.e. acetate (Jessen, 1995). In the presence of oxygen, metabolites other than those found in anaerobic conditions may be observed. Despite the formation of hydrogen peroxide, which may be formed during the aerobic metabolism of glucose, the yield of lactic acid, acetic acid, acetoin and ethanol are affected. *Lactobacillus plantarum*, which under anaerobic conditions mainly forms lactic acid from glucose, shows a dramatic increase in the production of acetic acid under aerobic conditions, together with small amounts of acetoin (Kröckel, 1995).

Proteolytic system of lactobacilli

Proteolysis

Proteolysis is considered the most complex of the three primary events during food fermentations, the other two being carbohydrate fermentation and lipolysis. It is a general belief that lactic acid bacteria have limited abilities to synthesize amino acids, which are essential for their growth, and most raw food materials contain insufficient amounts of free amino acids and low molecular mass peptides to sustain growth (Law and Kolstadt, 1983; Thomas and Pritchard, 1987). Although they are considered as weak proteolytic bacteria compared with other groups of microorganisms, it has be shown that lactic acid bacteria posses a complex proteolytic system capable of hydrolysing food proteins to peptides and amino acids (Kunji *et al.*, 1996; Mierau *et al.*, 1997). Furthermore, it is generally accepted that their proteolytic system contributes to the degradation of food protein and hence to the texture, taste and aroma of fermented products (McSweeney and Sousa, 2000).

The most extensively studied proteolytic system is that of *Lactococcus lactis*, and it serves as a model for all lactic acid bacteria. The second best unravelled proteolytic systems are those of *Lactobacillus* species, most notably *Lactobacillus helveticus*, *Lactobacillus bulgaricus* and *L. casei*. An extracellular membrane-anchored serine proteinase (PrtP) is an essential component of this system. PrtP exists in at least two variants with somewhat different specificities in the degradation of milk casein. The gene encoding PrtP has been cloned and sequenced for a number of *Lactobacillus paracasei* (Holck and Nes, 1992) and *L. bulgaricus* (Gilbert *et al.*, 1996) strains. The *L. paracasei* enzyme shows more than 95% similarity to the lactococcal one, while the *L. delbrueckii* proteinase shows up to 40% identity over the first 820 residues when compared to the lactococcal enzymes; however the C-terminal part does not share any homology. Studies have indicated that *L. helveticus* may contain two proteinases with different substrate specificities (Gilbert *et al.*, 1997), while a cell envelope-associated proteinase gene (prtH) was identified in *L. helveticus* CNRZ32, with a deduced amino acid sequence having significant identity (45%) to that of the lactococcal PrtP proteinases (Pederson *et al.*, 1999).

The majority of sourdough lactic acid bacteria does not exhibit cell wall-associated proteinase activity (Pepe *et al.*, 2003; Vermeulen *et al.*, 2005). Generally, a comparable extent of protein degradation is observed in wheat sourdough and in chemically acidified dough (Thiele *et al.*, 2004; Loponen *et al.*, 2004). However, several strains of sourdough lactic acid bacteria strains exhibiting proteolytic activity were characterized (Gobbetti *et al.*, 1996a; Di Cagno *et al.*, 2002; Pepe *et al.*, 2003) and a contribution of selected lactic acid bacteria to proteolysis could be demonstrated by analysis of the degradation of albumins, globulins, and gliadins in wheat sourdoughs (Di Cagno *et al.*, 2002; Pepe *et al.*,

2003; Zotta *et al.*, 2006). The analysis of peptide and amino acid levels in wheat sourdoughs indicate that *L. sanfranciscensis* preferably utilizes peptides during growth in sourdough (Thiele *et al.*, 2004). Comparable to *Lactococcus lactis* and *L. plantarum*, *L. sanfranciscensis* expresses transport systems for oligo- and dipeptides (Vermeulen *et al.*, 2005) and peptides are hydrolysed by intracellular peptidases, several of which have been characterized at the biochemical or genetic level (Gobbetti *et al.*, 1996a; Gallo *et al.*, 2005; Vermeulen *et al.*, 2005). Analysis of the regulation of peptide uptake systems and peptidases during growth of *L. sanfranciscensis* has shown that genes coding for the peptide uptake systems for dipeptides (DtpT) (Foucaud *et al.*, 1995) and an oligopeptide transport system (Opp) were expressed during exponential growth in sourdough and their expression was reduced in stationary phase cells or when the peptide supply in dough was increased (Vermeulen *et al.*, 2005).

Amino acid transport

In lactococci, the products of the initial casein degradation (amino acids and peptides) are transported into the cell by transport systems specific for amino acids (Konings *et al.*, 1989), two di- and tripeptides (DtpT and DtpP) (Foucaud *et al.*, 1995) and an oligopeptide transport system (Opp) accepting four to eight residue peptides (Tynkkynen *et al.*, 1993). However, little information is available on the transport of casein breakdown products in lactobacilli. Results suggest that the amino acid transport systems in *L. helveticus* are similar to the lactococcal (Nakajima *et al.*, 1998). The gene coding for a branched chain amino acid carrier (*brnC*) of *L. delbrueckii* subsp. *lactis* has been cloned and sequenced (Stucky *et al.*, 1995), and it is driven by the proton motive force. For the same organism the genes coding for an aromatic acid and a dipeptide transporter, *aroP* and *dppE*, respectively, have also been cloned and sequenced (Kunji *et al.*, 1996). For *L. helveticus*, a homologue of DtpT is specified by a sequence located downstream of pepN (Christensen *et al.*, 1995), and transport experiments have shown that substrates, typical for the lactococcal DtpT, are indeed transported by this organism. Experiments also indicate that an oligopeptide transport system is present in *L. helveticus* (Nakajiama *et al.*, 1998). Inside the cell, several peptidases with a wide range of specificity complete the degradation (Christensen *et al.*, 1999).

Amino acid metabolism

In addition to proteolysis, bacterial amino acid metabolism contributes to flavour formation during fermentation. In recent years, it has become clear that a number of enzymes are involved in the conversion of amino acids to flavour components. Indeed, the genome sequence analysis of several species of lactic acid bacteria provided insight into the metabolic pathways for amino acid conversion (van Kranenburg *et al.*, 2002). These enzymes may catalyse reactions such as transamination, deamination, decarboxylation, and cleavage of the amino acid side chain. Branched-chain amino acids can be transaminated to ketoacids, which then undergo either spontaneous degradation or they are enzymatically converted to the corresponding aldehydes or carboxylic acids (Smit *et al.*, 2000). Amino acid transamination is a key step in the amino acid conversion to aroma compounds by lactic acid bacteria. Indeed, in lactic acid bacteria catabolism of AtAAs, BcAAs and Met is essentially initiated by a transamination reaction since the degradation occurs only in presence of an alpha-ketoacid which is used as amino group acceptor. This was demonstrated in mesophilic lactobacilli such as *L. paracasei*, *L. casei*, *L. plantarum*, *Lactobacillus rhamnosus* (Gummalla and Broadbent, 1996; Tammam *et al.*, 2000) and also in thermophilic lactobacilli such as *L. helveticus*, *L. delbrueckii* subsp. *lactis*, and *L. delbrueckii* subsp. *bulgaricus* (Gummalla and Broadbent, 1999). α-Ketoglutarate serves as amino acceptor in the transamination reaction of leucine, phenylalanine and other amino acids, and the addition of α-ketoglutarate strongly increases amino acid conversion of *Lactobacillus sakei* and *L. plantarum* (Yvon *et al.*, 1998; Larrouture *et al.*, 2000). Lactic acid bacteria exhibit glutamate dehydrogenase activity in a strain specific manner. The enzyme catalyses the NAD(P)H-dependent recycling of glutamate to α-ketoglutarate, and consequently increases the flux through the

transaminase reaction (Gänzle *et al.*, 2007). The catabolism of leucine and phenylalanine was analysed in detail with strains of *L. sakei* and *L. plantarum* (Groot and de Bont, 1998; Larrouture *et al.*, 2000), valine and isoleucine are degraded by comparable metabolic pathways. Cystathionine lyase (Cxl) is a key enzyme in the metabolism of methionine and cysteine in lactic acid bacteria Cystathionine-γ-lyase was purified and characterized from *L. fermentum* and *L. reuteri* (De Angelis *et al.*, 2002). Vermeulen *et al.* (2003) reported that *L. fermentum*, *L. reuteri*, *L. pontis*, *Lactobacillus panis* and *Lactobacillus mindensis* but not *L. sanfranciscensis*, *L. plantarum* and *Lactobacillus brevis* expressed genes coding for Cxl. Cxl activity of lactic acid bacteria contributes to the flavour development during cheese ripening (Weimer *et al.*, 1999), and a cysteine uptake system was shown to be essential for oxygen tolerance in *L. fermentum* (Turner *et al.*, 1999) but a possible functional role of cysteine and methionine metabolism in sourdough remains to be determined.

Lipolytic system of lactobacilli

Lipolysis is among the principal events occurring during cheese ripening. Free fatty acids can be further converted to methyl ketones, lactones, thioesters, keto and hydroxy acids, which contribute in addition to the free fatty acids to the flavour of the ripened product, while the volatile short chain fatty acids are responsible for the rancid flavour of milk (El Soda *et al.*, 1995). The main lipolytic agents in cheese include the indigenous milk lipoprotein lipase, but also the lipases and esterases produced by the starter and non-starter bacteria, and depending on the cheese variety enzyme preparations added during manufacturing.

To hydrolyse milk fat in milk and cheese, lactic acid bacteria possess esterolytic and lipolytic enzymes capable of hydrolysing a range of esters of FFA, tri-, di, and monoacylglyceride substrates (Fox and Wallace, 1997). Despite the presence of these enzymes, lactic acid bacteria, especially *Lactococcus* and *Lactobacillus* spp. are generally considered to be weakly lipolytic in comparison to species such as *Pseudomonas*, *Acinetobacter* and *Flavobacterium* (Fox *et al.*, 1993). However, because of their presence in cheese at high numbers over an extended ripening period, lactic acid bacteria are considered likely to be responsible for the liberation of significant levels of FFA. To date, lipases/esterases of lactic acid bacteria appear to be exclusively intracellular and a number have been identified and characterized (Chich *et al.*, 1997; Castillo *et al.*, 1999; Liu *et al.*, 2001). El-Soda *et al.* (1986) found intracellular esterolytic activities against substrates up to C5:0 in *L. helveticus*, *L. delbrueckii* subsp. *bulgaricus*, *L. delbrueckii* subsp. *lactis* and *L. acidophilus*, with *L. delbrueckii* subsp. *lactis* and *L. acidophilus* displaying the highest activities. Khalid and Marth (1990) reported the quantitative estimation of the lipolytic activity of *L. casei*, *L. plantarum* and *L. helveticus* towards milk fat, olive oil and tributanoic acid emulsions. The three emulsions were hydrolysed by the lactobacilli with the exception of one *L. casei* strain, which failed to hydrolyse olive oil. According to Lee and Lee (1990), esterolytic and lipolytic enzymes were produced by cell lysis of *L. casei* subsp. *casei* LLG, while *L. fermentum* contains a cell surface-associated esterase specific for C4:0, which can hydrolyse β-naphthyl esters of fatty acids from C2:0 to C10:0 (Gobbetti *et al.*, 1997). Gobbetti *et al.* (1996b) reported the purification of an intracellular lipase from a *L. plantarum* strain isolated from Cheddar cheese, with a molecular mass of 65 kDa, and pH and temperature optima of 7.5 and 35°C, respectively. The enzyme was relatively heat stable to a temperature of 65°C but was irreversibly inactivated on heating to 75°C for 2 min. Hydrolysis of triacylglycerides indicated that the enzyme had highest activity on tributanoic acid, less activity on tridodecanoic and trihexadecanoic acids and no activity on tri-cis-9-octadecenoic acid.

Lipids are only a minor component of wheat and rye flours but have a significant effect on bread quality. *L. sanfranciscensis* is auxotroph for unsaturated fatty acids (Sugihara and Kline, 1975). Unsaturated fatty acids are subject to autoxidation during flour storage, and are oxidized by cereal lipoxygenase activity during dough mixing (Laignelet and Dumas, 1984). (E)-2-Nonanal and other aldehydes resulting from lipid oxidation are key aroma compounds in wheat

and rye bread that impart a 'fatty', 'metallic' or 'green' flavour (Hansen and Schieberle, 2005) and the concentrations of these flavour compounds are significantly reduced during sourdough fermentation (Czerny and Schieberle, 2002). The SC-ADH activity of lactobacilli contributes to the reduction of these flavour compounds during sourdough fermentation, yeasts additionally may exhibit ADH activity in dough.

The taxonomy of the genus *Lactobacillus*

Some taxonomic background

The genus *Lactobacillus* belongs to the large group of lactic acid bacteria, which are all Gram-positive non-sporing cocci, coccobacilli or rods, having a DNA base composition of less than 50 mol% G + C. As mentioned before they lack catalase and need a fermentable carbohydrate for growth. The lactic acid bacteria in the broad sense comprise genera such as *Aerococcus, Alloiococcus, Atopobium, Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Oenococcus, Paralactobacillus, Pediococcus, Streptococcus, Tetragenococcus, Vagococcus* and *Weissella*. The genus *Bifidobacterium, Gardnerella, Scardovia* and *Parascardovia* are often also included in this collection, although phylogenetically they belong to the Actinobacteria subdivision (PHYLUM 3), of the Gram-positive Eubacteria (the Firmicutes), comprising also *Propionibacterium, Brevibacterium* and the microbacteria. The latter taxa are only very distantly related to the genuine lactic acid bacteria.

The genus *Lactobacillus* belongs phylogenetically to the phylum *Firmicutes* (Garrity *et al.*, 2004). The family *Lactobacillaceae* comprises the main family in the order *Lactobacillales* which itself belongs to the class *Bacilli*. From the other members of the family mentioned above, the genera *Paralactobacillus* and *Pediococcus* are most noteworthy since species of these genera tend to intermingle phylogenetically with the variety of species of the genus *Lactobacillus*.

This classification was mainly build on the results of 16S rRNA sequence analysis (*Taxonomic Outline of the Procaryotes*; Garrity *et al.*, 2004), and as mentioned before does not necessarily reflect the metabolic diversity discussed above. Equally essential as the 16S rRNA sequencing technique to this scheme, is the species concept (*The ad hoc committee for the re-evaluation of the species definition in bacteriology*; Stackebrandt *et al.*, 2002). Although the species concept remains subject of animated debates among taxonomists (Rosselló-Mora and Amann, 2001; Rosselló-Mora, 2003; Gevers *et al.*, 2005), it remains the formal unit of bacterial classification and is defined as 'a monophyletic and genomically coherent cluster of individual organisms that show a high degree of overall similarity with respect to many independent characteristics,' and 'is diagnosable by a discriminative phenotypic property' (Rosselló-Mora and Amann, 2001). This phylophenetic concept requires that besides genetic evidence, some phenotypic characteristics will discriminate a possible new species from its closest phylogenetic neighbours. When phenotypic variation in a species is considerable, a species may be further subdivided into subspecies, based on this phenotypic variation; subspecies can but need not be supported by genetic determinants (Rosselló-Mora and Amann, 2001).

In practice, a species is defined by two main genotypic criteria: strains with a total DNA similarity of 70% or higher (relative binding in a hybrid DNA reassociation experiment) and a difference in the melting temperature (ΔT_m) equal to or lower than 5°C, will be considered to belong to a single species; in addition the 16S rRNA gene sequence similarity should not differ more than 3%. As mentioned above, phenotypic features should be sought that confirm the proposed groupings.

DNA–DNA similarity measures are thus still considered the gold standard technique for the delineation of bacterial species. Since this technique is laborious, it is very unpractical for the 113 species of the genus *Lactobacillus*. Therefore, the closest phylogenetic species are often identified by a (partial) 16S rRNA sequencing, which after comparison with the large collection of 16S rRNA sequences available in public databases, allows the identification of the most closely related species for which DNA–DNA hybridizations will need to be set up.

For several reasons, 16S rRNA gene sequencing can never be used as the sole method for species delineation (Stackebrandt and Goebel, 1994).

1 A first limit might be that often a single representative strain per species is used for the 16S rRNA analysis, lacking the possibility to position a new isolate in the biological diversity of the species considered.
2 The use of partial sequences is also making the result of 16S rRNA sequences less reliable.
3 The many sequencing errors present in the reference sequences (often from the early days of sequencing), will also influence the final tree.
4 Sequence alignment, essential for sequence similarity calculation, is a highly subjective business. Not only does it rely on a wealth of algorithms, but often manual editing is necessary to 'improve' the result obtained. Critical are the ease one allows the software to create gaps, and the 'cost' defined to extend these gaps.
5 Also, in the pairwise calculation of the sequence similarities, gaps can be included or not and phylogenetic corrections can be applied or not.
6 The cluster algorithm chosen, as well as the selection of reference sequences included, will also affect the shape of the final tree.
7 Finally, as a conserved taxonomic marker, 16S rRNA is not really suitable to study small differences between closely related species.

For these reasons, 16S rRNA sequencing will be useful to frame a new isolate in a well-known phylogenetic scheme, but may not solve the real identification or classification problem. A polyphasic approach (Vandamme *et al.*, 1996a), taking into consideration a variety of information sources, should result in a more reliable identification or classification.

In view of the wide use of lactic acid bacteria in food applications, identification and classification, however, are very important. The discussion whether evolutionary deductions should automatically reflect on nomenclatural designation (Dellaglio *et al.*, 2004b) is a very relevant one. Nomenclature is essential for proper food labelling and will allow producers to communicate in a formal way about the bacteria they add to foods. Safety aspects, for example, have also been linked to species definition (the QPS principle; http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620759439.htm). One could therefore support the automatic link between evolution and speciation (de Quieroz and Gauthier, 1992; Woese, 1998; Cantino 1999), but its automatic translation into nomenclatural designations can be questioned for practical and other reasons.

As an example we could use the *Lactobacillus casei* case. The taxonomic controversy has been going on for quite some time. *Lactobacillus casei* was described by Orla-Jensen in 1919. Hansen and Lessel (1971) choose strain ATCC 393 as the neotype strain based on a limited number of phenotypic traits. DNA–DNA hybridization experiments (Dellaglio *et al.*, 1975) showed that strain ATCC 393^T had high DNA similarity with the former type strain of '*Lactobacterium zeae*' (Kuznetsov, 1959) and as such was shown not the best neotype strain for *L. casei*. Using DNA–DNA hybridization experiments Collins *et al.* (1989b) confirmed the separate position of strain ATCC 393^T and transferred all other *L. casei* strains to a new species *L. paracasei*.

In a first request for opinion, Dellaglio *et al.* (1991) proposed strain *Lactobacillus casei* ATCC 334 (not investigated by Collins *et al.*, 1989b), as an alternative neotype strain of *L. casei* in place of ATCC 393^T and requested the rejection of the name *L. paracasei*. This request was denied by the Judicial Commission of the International Committee on Systematic Bacteriology (Wayne, 1994), as this would create a precedent in replacing a type strain that was officially described and which was still readily available (Wayne, 1994). In 1996, Dicks *et al.* reclassified *L. casei* subsp. '*casei*' ATCC 393^T and '*Lactobacterium zeae*' ATCC 15820 as *L. zeae* nom. rev., and designated strain ATCC 334 as the neotype of *L. casei* subsp. '*casei*'. They formerly rejected the name *L. paracasei*. Since this species, however, was validly published, the situation was unclear, as both proposals were standing and a new opinion of the Judicial Commission was required. The